

What Is Claimed Is:

X 1. An isolated polypeptide comprising the amino acid sequence of SEQ ID NO:2.

DAB/BS 2. An isolated polynucleotide molecule comprising a nucleotide sequence encoding the polypeptide sequence of claim 1.

3. The isolated polynucleotide molecule of claim 2 comprising a nucleic acid having the sequence of SEQ ID NO:1.

4. A vector comprising the isolated polynucleotide molecule of claim 2.

5. A host cell comprising the vector of claim 4.

6. A method comprising:

(a) transforming a *Corynebacterium* species host cell with the polynucleotide molecule of claim 2; wherein said isolated polynucleotide molecule is integrated into said host cell's chromosome thereby increasing the total number of said amino acid biosynthetic pathway genes in said host cell chromosome, and

(b) selecting a transformed host cell.

7. The method of claim 6 further comprising screening for increased amino acid production.

8. The method of claim 6 wherein said polynucleotide molecule further comprises at least one of the following:

(a) a nucleic acid molecule encoding a *Corynebacterium species* lysine pathway *asd* amino acid sequence.

- (b) a nucleic acid molecule encoding a *Corynebacterium species* lysine pathway *dapA* amino acid sequence;
- (c) a nucleic acid molecule encoding a *Corynebacterium species* lysine pathway *dapB* amino acid sequence;
- 5 (d) a nucleic acid molecule encoding a *Corynebacterium species* lysine pathway *ddh* amino acid sequence; and
- (e) a nucleic acid molecule encoding a *Corynebacterium species* lysine pathway *'lysA* amino acid sequence;
- 10 (f) a nucleic acid molecule encoding a *Corynebacterium species* lysine pathway *lysA* amino acid sequence; and
- (g) a nucleic acid molecule encoding a *Corynebacterium species* lysine pathway *ORF2* amino acid sequence.

9. The method of claim 8 further comprising screening for increased amino acid production.

10. The method of claim 6, wherein said isolated polynucleotide molecule further comprises at least one of the following:

- 15 (a) a nucleic acid molecule encoding the *asd* amino acid sequence of SEQ ID NO:4;
- (b) a nucleic acid molecule encoding the *dapA* amino acid sequence of SEQ ID NO:6;
- 20 (c) a nucleic acid molecule encoding the *dapB* amino acid sequence of SEQ ID NO:8;
- (d) a nucleic acid molecule encoding the *ddh* amino acid sequence of SEQ ID NO:10;
- 25 (e) a nucleic acid molecule encoding the *'lysA* amino acid sequence of SEQ ID NO:21;
- (f) a nucleic acid molecule encoding the *lysA* amino acid sequence of SEQ ID NO:14;

CONTINUATION-IN-CHIEF-CASE

15
part B
16

(g) a nucleic acid molecule encoding the *ORF2* amino acid sequence of SEQ ID NO:16

11. The method of claim 6, wherein said isolated polynucleotide molecule further comprises the following:

- 5 (a) a nucleic acid molecule encoding the *asd* amino acid sequence of SEQ ID NO:4;
- (b) a nucleic acid molecule encoding the *dapA* amino acid sequence of SEQ ID NO:6;
- 10 (c) a nucleic acid molecule encoding the *dapB* amino acid sequence of SEQ ID NO:8; and
- (d) a nucleic acid molecule encoding the *ORF2* amino acid sequence of SEQ ID NO:16.

12. The method of claim 6, wherein said isolated polynucleotide molecule further comprises the following:

- 15 (a) a nucleic acid molecule encoding the *asd* amino acid sequence of SEQ ID NO:4;
- (b) a nucleic acid molecule encoding the *dapA* amino acid sequence of SEQ ID NO:6;
- 20 (c) a nucleic acid molecule encoding the *dapB* amino acid sequence of SEQ ID NO:8;
- (d) a nucleic acid molecule encoding the *ddh* amino acid sequence of SEQ ID NO:10; and
- (e) a nucleic acid molecule encoding the *ORF2* amino acid sequence of SEQ ID NO:16.

25 13. The method of claim 6, wherein said isolated polynucleotide molecule further comprises the following:

- (a) a nucleic acid molecule encoding the *asd* amino acid sequence of SEQ ID NO:4;

(b) a nucleic acid molecule encoding the *dapA* amino acid sequence of SEQ ID NO:6;

(c) a nucleic acid molecule encoding the *dapB* amino acid sequence of SEQ ID NO:8;

5 (d) a nucleic acid molecule encoding the *ddh* amino acid sequence of SEQ ID NO:10;

(e) a nucleic acid molecule encoding the *lysA* amino acid sequence of SEQ ID NO:21; and

10 (f) a nucleic acid molecule encoding the *ORF2* amino acid sequence of SEQ ID NO:16.

14. The method of claim 6, wherein said isolated polynucleotide molecule further comprises the following:

(a) a nucleic acid molecule encoding the *asd* amino acid sequence of SEQ ID NO:4;

15 (b) a nucleic acid molecule encoding the *dapA* amino acid sequence of SEQ ID NO:6;

(c) a nucleic acid molecule encoding the *dapB* amino acid sequence of SEQ ID NO:8;

20 (d) a nucleic acid molecule encoding the *ddh* amino acid sequence of SEQ ID NO:10;

(e) a nucleic acid molecule encoding the *lysA* amino acid sequence of SEQ ID NO:14; and

(f) a nucleic acid molecule encoding the *ORF2* amino acid sequence of SEQ ID NO:16.

25 15. The method of claim 6 further comprising:

(a) growing said transformed host cell in a medium; and

(b) purifying an amino acid produced by said transformed host cell.

16. An isolated polynucleotide molecule comprising:
- (a) the polynucleotide molecule of claim 2; and
- (b) at least one additional *Corynebacterium* species lysine pathway gene selected from the group consisting of:
- (i) a nucleic acid molecule encoding an *asd* polypeptide;
- (ii) a nucleic acid molecule encoding a *dapA* polypeptide;
- (iii) a nucleic acid molecule encoding a *dapB* polypeptide;
- (iv) a nucleic acid molecule encoding a *ddh* polypeptide;
- (v) a nucleic acid molecule encoding a '*lysA*' polypeptide;
- (vi) a nucleic acid molecule encoding a *lysA* polypeptide; and
- (vii) a nucleic acid molecule encoding an *ORF2* polypeptide.
- Du C 2*
- 5
- 10

17. The isolated nucleic acid molecule of claim 16, wherein:
- (a) said *asd* polypeptide is SEQ ID NO:4;
- (b) said *dapA* polypeptide is SEQ ID NO:6;
- (c) said *dapB* polypeptide is SEQ ID NO:8;
- (d) said *ddh* polypeptide is SEQ ID NO:10;
- (e) said '*lysA*' polypeptide is SEQ ID NO:21;
- (f) said *lysA* polypeptide is SEQ ID NO:14; and
- (g) said *ORF2* polypeptide is SEQ ID NO:16.
18. An isolated polynucleotide molecule comprising:
- (a) the polynucleotide molecule of claim 2;
- (b) a nucleic acid molecule encoding the *asd* amino acid sequence of SEQ ID NO:4;
- (c) a nucleic acid molecule encoding the *dapA* amino acid sequence of SEQ ID NO:6;
- (d) a nucleic acid molecule encoding the *dapB* amino acid sequence of SEQ ID NO:8; and
- (e) a nucleic acid molecule encoding the *ORF2* amino acid sequence of SEQ ID NO:16.
- 15
- 20
- 25

19. An isolated polynucleotide molecule comprising:
- (a) the polynucleotide molecule of claim 2;
 - (b) a nucleic acid molecule encoding the *asd* amino acid sequence of SEQ ID NO:4;
 - 5 (c) a nucleic acid molecule encoding the *dapA* amino acid sequence of SEQ ID NO:6;
 - (d) a nucleic acid molecule encoding the *dapB* amino acid sequence of SEQ ID NO:8;
 - (e) a nucleic acid molecule encoding the *ddh* amino acid sequence of SEQ ID NO:10; and
 - 10 (f) a nucleic acid molecule encoding the *ORF2* amino acid sequence of SEQ ID NO:16.
20. An isolated polynucleotide molecule comprising:
- (a) the polynucleotide molecule of claim 2;
 - (b) a nucleic acid molecule encoding the *asd* amino acid sequence of SEQ ID NO:4;
 - 15 (c) a nucleic acid molecule encoding the *dapA* amino acid sequence of SEQ ID NO:6;
 - (d) a nucleic acid molecule encoding the *dapB* amino acid sequence of SEQ ID NO:8;
 - (e) a nucleic acid molecule encoding the *ddh* amino acid sequence of SEQ ID NO:10;
 - 20 (f) a nucleic acid molecule encoding the *'lysA* amino acid sequence of SEQ ID NO:21; and
 - (g) a nucleic acid molecule encoding the *ORF2* amino acid sequence of SEQ ID NO:16.

DRAFT - 11/20/2018

21. An isolated polynucleotide molecule comprising:
- (a) the polynucleotide molecule of claim 2;
 - (b) a nucleic acid molecule encoding the *asd* amino acid sequence of SEQ ID NO:4;
 - 5 (c) a nucleic acid molecule encoding the *dapA* amino acid sequence of SEQ ID NO:6;
 - (d) a nucleic acid molecule encoding the *dapB* amino acid sequence of SEQ ID NO:8;
 - (e) a nucleic acid molecule encoding the *ddh* amino acid sequence of SEQ ID NO:10;
 - 10 (f) a nucleic acid molecule encoding the *lysA* amino acid sequence of SEQ ID NO:14; and
 - (g) a nucleic acid molecule encoding the *ORF2* amino acid sequence of SEQ ID NO:16.
- 15 22. The isolated polynucleotide molecule of claim 18 comprising pK184-KDAB.
23. The isolated polynucleotide molecule of claim 20 comprising pD11-KDABH'L.
- 20 24. The isolated polynucleotide molecule of claim 21 comprising pD2-KDABHL.
25. A vector comprising the polynucleotide molecule of claim 16.
26. A host cell comprising the vector of claim 25.

Sue B17

27. The host cell of claim 26 wherein said host cell is a *Brevibacterium* selected from the group consisting of *Brevibacterium flavum* NRRL-B30218, *Brevibacterium flavum* NRRL-B30219, *Brevibacterium lactofermentum*

*Sent
B7*

NRRL-B30220, *Brevibacterium lactofermentum* NRRL-B30221, *Brevibacterium lactofermentum* NRRL-B30222, *Brevibacterium flavum* NRRL-30234 and *Brevibacterium lactofermentum* NRRL-30235.

28. The host cell of claim 26 wherein said host cell is *Escherichia coli* DH5⁵ α MCR NRRL-B30228.

29. The host cell of claim 26 wherein said host cell is a *C. glutamicum* selected from the group consisting of *C. glutamicum* NRRL-B30236 and *C. glutamicum* NRRL-B30237.

30. A method of producing lysine comprising culturing the host cells of claim 5 wherein said host cells comprise one or more of:

(a) increased enzyme activity of one or more lysine biosynthetic pathway enzymes compared to the genetically unaltered host cell;

(b) one or more copies of each gene encoding a lysine biosynthetic pathway enzyme; and,

(c) alteration of one or more transcription factors regulating transcription of one or more genes encoding a lysine biosynthetic pathway enzyme, wherein said host cell produces lysine in said culture medium.

31. The method of claim 30 wherein said increased enzyme activity comprises overexpressing one or more genes encoding one or more lysine biosynthetic pathway enzymes.

32. The method of claim 31 wherein said one or more genes are operably linked directly or indirectly to one or more promoter sequences.

33. The method of claim 32 wherein said operably linked promoter sequences are heterologous, endogenous, or hybrid.

34. The method of claim 33 wherein said promoter sequences are one or more of: a promoter sequence from the 5' end of genes endogenous to *C. glutamicum*, a promoter sequence from plasmids that replicate in *C. glutamicum*, and, a promoter sequence from the genome of phage which infect *C. glutamicum*.
- 5 35. The method of claim 33 or 34 wherein one or more of said promoter sequences are modified.
- 10 36. The method of claim 35 wherein said modification comprises truncation at the 5' end, truncation at the 3' end, non-terminal insertion of one or more nucleotides, non-terminal deletion of one or more nucleotides, addition of one or more nucleotides at the 5' end, addition of one or more nucleotides at the 3' end, and, combinations thereof.
- 15 37. The method of claim 30 wherein said increased enzyme activity results from the activity of one or more modified lysine biosynthetic pathway enzymes wherein said enzyme modification results in a change in kinetic parameters, allosteric regulation, or both, compared to the enzyme lacking said modification.
38. The method of claim 37 wherein said change in kinetic parameters is a change in K_m , V_{max} or both.
- 17 39. The method of claim 37 wherein said change in allosteric regulation is a change in one or more enzyme allosteric regulatory sites.
- 20 40. The method of claim 37 wherein said modification is a result of a change in the nucleotide sequence encoding said enzyme.
41. The method of claim 40 wherein said change in said nucleotide sequence is an addition, insertion, deletion, substitution, or a combination thereof, of one or more nucleotides.

42. The method of claim 30 wherein said alteration of one or more transcription factors comprises one or more mutations in transcription inhibitor proteins, one or more mutations in transcription activator proteins, or both, wherein said one or more mutations increases transcription of the target nucleotide sequence compared to the transcription by said one or more transcription factors lacking said alteration.

5
43. The method of claim 42 wherein said one or more mutations is a change in said nucleotide sequence encoding said transcription factor.

10
44. The method of claim 43 wherein said change in said nucleotide sequence is an addition, insertion, deletion, substitution, or a combination thereof, of one or more nucleotide.

15
45. An isolated polypeptide, wherein said polypeptide comprises an amino acid sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO:19.

46. The polypeptide of claim 45, wherein said polypeptide has the amino acid sequence of SEQ ID NO:19.

20
47. An isolated polynucleotide molecule comprising a nucleotide sequence encoding the polypeptide of claim 45.

48. The isolated polynucleotide molecule of claim 47 comprising a nucleic acid having the sequence of SEQ ID NO:18.

49. A vector comprising the polynucleotide molecule of claim 47.

50. A host cell comprising the vector of claim 49.

51. The host cell of claim 50 wherein said host cell is NRRL B30360.

52. A method comprising:

- (a) transforming a *Corynebacterium* species host cell with the polynucleotide molecule of claim 47, and
(b) selecting a transformed host cell.

5 53. An isolated polypeptide wherein said polypeptide comprises a polypeptide having at least 95% sequence identity to the amino acid sequence of SEQ ID NO:21.

10 54. The polypeptide of claim 53 having the amino acid sequence of SEQ ID NO:21.

55. An isolated polynucleotide molecule comprising a nucleotide sequence encoding the polypeptide of claim 53.

15 56. The isolated polynucleotide molecule of claim 55 comprising a nucleic acid having the sequence of SEQ ID NO: 20.

57. A vector comprising the polynucleotide molecule of claim 55.

15 58. A host cell comprising the vector of claim 57.

59. The host cell of claim 58 wherein said host cell is selected from the group consisting of NRRL B30218, NRRL B30220 and NRRL B30222.

20 60. A method comprising:

- (a) transforming a *Corynebacterium* species host cell with the polynucleotide molecule of claim 55, and
(b) selecting a transformed host cell.

Sue B 18

61. The isolated polynucleotide molecule of claim 2 further comprising a promoter sequence where said promoter sequence has at least 95% sequence identity to SEQ ID NO:17.

5 62. The polynucleotide of claim 61 where said promoter sequence has the nucleotide sequence of SEQ ID NO: 17.

Sue B 19

63. The isolated polynucleotide molecule of claim 61 wherein said promoter is operably directly linked to the LysA gene.

64. A vector comprising the isolated polynucleotide of claim 61.

65. A host cell comprising the vector of claim 64.

10 66. The host cell of claim 65 wherein said host cell is NRRL B30359.

67. A method comprising:

- (a) transforming a *Corynebacterium* species host cell with the polynucleotide molecule of claim 61, and
- (b) selecting a transformed host cell.

Add C4